ORAL CONTRIBUTIONS

807 Novel Approaches to Cardiovascular Gene Transfer and Targeting

Monday, March 18, 2002, 9:15 a.m.-10:30 a.m.
Georgia World Congress Center, Room 360W

9:15 a.m.

807-1 Intraoperative Cell-Cycle Blockade Therapy Using E2F Decoy for Prevention of Early Atherosclerosis in Coronary Bypass Vein Grafts: A Volumetric IVUS Subanalysis From a Prospective Randomized Double Blind Clinical Trial

Mitsuyasu Terashima, Eberhard Grube, Takefumi Takahashi, Hideaki Kaneda, Thomas Felderhoff, Stein Iversen, Paul G. Yock, Yasuhiro Honda, Peter J. Fitzgerald, *Stanford University, Stanford, California, Heart Center Siegburg, Siegburg, Germany.*

Background: Previous experimental and peripheral studies of vein grafts have shown that *ex vivo* cell-cycle blockade therapy using E2F Decoy inhibits neointimal hyperplasia and subsequent accelerated atherosclerosis -- the primary factors in late graft failure. The aim of this study was to investigate the safety and efficacy of intraoperative cell-cycle blockade therapy on human coronary saphenous vein bypass grafts (SVG) using volumetric intravascular ultrasound (IVUS).
Methods: Decoy oligodeoxynucleotide, which binds and inactivates the pivotal cell-cycle transcription factor E2F, was delivered to the tissue *ex vivo* using a transfection device under completely non-distending conditions. Patients were assigned to either the treatment group (40 μM E2F decoy) or the placebo group (normal saline alone) in a prospective, randomized, and double-blind manner. IVUS was performed on a subset of grafts with automated pullback at 12 months following surgery. On the cross-sectional IVUS image, intimal area was measured as the area of an internal hypoechoic layer between the lumen/intimal border and the external border of hypoechoic layer (EHL). Volumetric IVUS analysis was performed over a 50-mm SVG segment (10 mm to 60 mm from the tip of guiding catheter or SVG orifice) using Simpson's method.
Results: Volumetric analysis was available in 65 grafts (35 E2F-decoy-treated : 30 placebo) at 12 months. Intimal volume/50mm and standardized intimal volume index (intimal volume / EHL volume, %) of the E2F group were significantly less than those of the placebo group (intimal volume/50mm: 78.6±45.6 mm^3 vs 114.8±78.3 mm^3, p=0.024, and standardized intimal volume index: 12.5±6.0% vs 18.4±10.1%, p=0.005). There was no difference in EHL-volume/50mm between the two groups. No adverse IVUS findings were observed in either the E2F or the placebo groups.
Conclusion: Volumetric IVUS analysis demonstrated that intraoperative cell-cycle blockade therapy with E2F Decoy significantly suppressed early neointimal hyperplasia of human coronary saphenous vein grafts. This new approach may contribute to reducing long-term morbidity and mortality following primary coronary bypass with saphenous vein grafts.

9:30 a.m.

807-2 A Tissue Engineered Stent for Cell-Based Vascular Gene Transfer

Carmelo J. Panetta, Katsumi Miyauchi, David Berry, Robert D. Simari, David R. Holmes, Robert S. Schwartz, Noel M. Caplice, *Mayo Foundation and Clinic, Rochester, Minnesota, Juntendo University, Tokyo, Japan.*

Background: Cell based gene transfer using a stent platform would provide significant advantages in terms of site-specific gene expression in the vasculature. The current study presents a novel stent design that allows stable in vivo transgene expression over a 4 week period in the vasculature.
Methods: A fiber matrix mesh was attached to Wiktor stent and coated with fibronectin prior to seeding with autologous porcine smooth muscle cells (SMC), which were stably transduced with a plasmid encoding green fluorescence protein (GFP). Cells were grown to confluence in the mesh-stent prior to deployment in the porcine coronary artery. Four animals were studied, each receiving one autologous seeded mesh-stent. After four weeks, quantitative coronary angiography (QCA) was performed on each animal prior to sacrifice. The coronary containing the mesh-stent was removed, a section was plastic embedded for hemotoxylin and eosin stain and the other section for fluorescent microscopy. The cells were removed from the vessel, and the number of GFP expressing cells were counted using flow cytometry.
Results: Fibronectin coating of the mesh allowed 20 fold greater seeding of cells compared no coating (4.9×10^5 +/- 0.4×10^5 vs. 0.2×10^5 +/- 0.03×10^5 cells/cm^2 mesh; <0.001, n=6). Stable in vivo GFP expression within the mesh stent was demonstrated four weeks after implantation in the porcine coronary artery by fluorescent microscopy and flow cytometry. No significant change in GFP positive cell number within the stent occurred in vivo when compared to pre-insertion analysis of a companion cell seeded mesh-stent (5.8×10^6 +/- 1.5×10^6 vs. 5.3×10^6 +/- 0.7×10^6 cells/cm^2 mesh; p=0.3). QCA revealed a maximal mean percent stenosis of 13.5 +/- 5.9 % (n=4) and histologic analysis revealed no apparent inflammatory infiltrate in the stented segment.
Conclusions: This is the first in vivo study to show stable cell-based gene transfer of large numbers of genetically engineered cells using a stent platform. These results have implications for gene therapy where long term delivery of transgene is a desirable therapeutic option.

9:45 a.m.

807-3 A New Approach for Inhibition of Neointimal Lesion Formation by Targeting Endothelin Expression Through CAAT/Enhancer Binding Protein Decoy Oligonucleotide

Ute Kelkenberg, Andreas H. Wagner, Jasmin Sarhaddar, Markus Hecker, Heiko E. von der Leyen, *Hannover Medical School, Hannover, Germany, University of Göttingen, Göttingen, Germany.*

Background: Deformation-induced synthesis of endothelin-1 (ET-1) may play a pivotal role in restenosis following percutaneous transluminal angioplasty. Thus, inhibition of ET-1-synthesis may be a potential promising therapeutic approach to treat restenosis. Since effective inhibitors of preproendothelin 1 processing to ET-1 are not available, we employed decoy oligonucleotides (dODN) to evaluate the effect of genetically engineered inhibition of ET-1 expression *in vivo*.
Methods: We analysed carotid arteries of hypercholesterolemic rabbits after balloon injury and incubation with therapeutic decoy (CAAT/enhancer binding protein [C/EBP] consensus dODN [10 μM, 30 min] or control decoys (C/EBP mutant dODN or buffer; n=7/group). Efficiency of decoy uptake was demonstrated using Texas-red labelled decoys.
Results: 2 days after transduction, C/EBP consensus decoy application resulted in significant reduction of C/EBP activity (electrophoretic mobility shift assay) and ET-1 expression (immunohistochemistry). 28 days post balloon injury we saw a significant reduction of intimal thickening (computerized morphometry; intima/media-ratio: 1.13 ± 0.22 [C/EBP consensus dODN] vs. 1.75 ± 0.45 [controls]; mean ± SEM, n = 7, p = 0.003). Macrophages were found throughout the intima of untreated and mutant decoy-dODN-transfected arteries corresponding to areas of macroscopic plaque. In the C/EBP consensus decoy-treated animals, only isolated macrophages in areas of small neointimal accumulation and no foam cells were observed (macrophage-positive area: 10.4 % ± 4.6 [C/EBP consensus dODN] vs. 17.2 % ± 4.9 [controls]; n = 7, p = 0.04).
Conclusion: These data indicate that intravascular application of a therapeutic decoy inhibiting C/EBP activity may be a feasible, safe, and effective method to reduce restenosis following balloon injury, especially under hypercholesterolemic conditions.

10:00 a.m.

807-4 Applicability of a Highly Efficient Electro-Gene Transfer Approach for the Human Heart

Luyi Sen, Guanggen Cui, Jack Judy, Hillel Laks, *UCLA Medical Center/UCLA School of Medicine, Los Angeles, California.*

Electroporation is a technique involving the application of short duration, high intensity electric field pulses to cells. It is commonly used for in vitro gene transfection of cell lines and primary cultures, but limited work has been reported in small animal organs and tissue. Recently, we have established a novel electroporation system for ex vivo gene transfer in large animal and human hearts. This device consisted of two electrode arrays directly in contact with the endocardium and epicardium in order to achieve uniform electroporation-enhanced gene transfer to the entire human heart. Using our recently developed rabbit heterotopic functional heart transplant model, we compared the efficiency of ex vivo intracoronary recombinant human interleukine 10 (IL-10) gene transfer, mediated by either electroporation (EP), liposome (LP) or adenovirus (AD). In EP group, the burst of electric pulses (pulse length 5 ms, number of pulse 10, burst-interval 2 min) was applied to the heart during and after ex vivo intracoronary gene infusion for 20 minutes. With 10mV/cm electric field strength, transgene was homogeneously transferred into the whole rabbit heart in 3-10 minutes. A significant increase in IL-10 mRNA level was observed 2 hours and reached a peak 3 days after gene transfer. In EP group, the transgene expression in the donor left ventricular myocardium was more than 5 times higher compared with that in the LP group, and 1.25 fold higher than that in AD group (n=8, p<0.01). The increase in transgene expression was paralleled with IL-10 protein expression. The distribution of the electroporation-mediated transgene expression was much more uniform than that in liposome-mediated gene transfer and adenovirus-mediated gene transfer. The hemodynamic and electrophysiologic parameters recorded from the donor heart were the same in EP group and the control group 6 days after transplantation. The arrhythmogenic effect was highest in AD group, less in LP group, and none in EP group. These results suggest that this new electroporation-mediated gene transfer is highly efficient and no significant cardiac adverse effect and is potentially applicable for ex vivo or in vivo gene delivery in human heart.

10:15 a.m.

807-5 Ultrasound in Conjunction With an Ultrasonic-Reflective Transfection Agent Enhances Gene Delivery to Cells

Shaoling Huang, David D. McPherson, Robert C. MacDonald, *Northwestern University, Chicago and Evanston, Illinois.*

Cationic liposomes have been developed that both provide ultrasonic enhancement and gene delivery to a target site. Low level ultrasound may enhance transfection, while retaining acoustic properties.
Methods: Liposomes consisting of cationic phospholipid (O-ethyldimyristoylphosphatidylcholine; EDMPC) and cholesterol were prepared according to a procedure previously described for anionic lipids and involving dispersion of the lipids in mannitol solution, lyophilization and rehydration. The liposomes were combined with DNA to form lipoplexes, which only slightly diminished ultrasound reflectivity. Cultured cells in 6-well plates were exposed to 1-Mhz ultrasound (0.5 W/cm^2, 30 sec) in the presence of the acoustic lipoplexes. Quantification of transfection efficiency was by -galactosidase expression and that of DNA uptake by fluorescence of YOYO-labeled -galactosidase plasmid (-gal DNA).
Results: Uptake of DNA into vascular smooth muscle cells and transfection of BHK cells